RANKL/OPG signaling affects the bone structure in rat model of mandibular osteoradionecrosis

A sinalização RANKL/OPG acomete estrutura óssea em modelo de rato de osteorradionecrose mandibular

DOI:10.34119/bjhrv5n1-199

Recebimento dos originais: 08/01/2021
Aceitação para publicação: 04/02/2022

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ABSTRACT
Objective: Osteoradionecrosis (ORN) of the jaws is an important side effect of head and neck cancer radiotherapy. The main objective of this work was to evaluate the expression of RANK, RANKL and OPG proteins, markers of bone remodeling, in rats submitted to radiotherapy. Material and Methods: Six-month-old male Wistar rats were divided into two groups: Irradiated group (IR), which received a single dose of 20 Gy in the jaw and after seven days had their three left mandibular molars extracted; Control group (C), which didn't receive radiation, but underwent the same procedures. Twenty one days after the dental extractions, the animals were euthanized and their mandibles were removed for analysis. The volume density (Vv) of immunoexpression of RANK/RANKL/OPG was quantified by stereology. The bone volume ratio (BV/TV), the trabecular thickness (Tb.Th), the trabecular separation (Tb.Sp) and the trabecular number (Tb.N) were analyzed by microtomography (Micro-CT). Results: Vv of immunoexpression of RANKL and OPG were, respectively, 53% higher and 50% lower in IR animals compared to C group ($p<0.05$). Micro-CT showed that in the IR animals there was a reduction of the bone mass compared to the C animals. BV/TV and Tb.Th were 33% and 38% lower, respectively, in the IR animals ($p<0.05$), while Tb.Sp was 37.5% higher in these animals ($p<0.05$). Conclusions: Ionizing irradiation in a single high dose, followed by dental extractions promoted ORN in mandibular bone in rats, together with changes in the markers of bone remodeling RANK and OPG.

Keywords: Radiotherapy, Osteoradionecrosis, Bone remodeling, RANKL, OPG.

RESUMO
Objetivo: A osteorradionecrose (ORN) das mandíbulas é um importante efeito colateral da radioterapia de câncer de cabeça e pescoço. O principal objetivo deste trabalho foi avaliar a expressão das proteínas RANK, RANKL e OPG, marcadores de remodelagem óssea, em ratos submetidos à radioterapia. Material e Métodos: Ratos Wistar machos de seis meses de idade foram divididos em dois grupos: grupo irradiado (IR), que recebeu uma dose única de 20 Gy na mandíbula e após sete dias teve seus três molares mandibulares esquerdos extraídos; Grupo controle (C), que não recebeu radiação, mas foi submetido aos mesmos procedimentos. Vinte e um dias após as extrações dentárias, os animais foram eutanizados e suas mandíbulas foram removidas para análise. A densidade de volume (Vv) da imunoexpressão de RANK/RANKL/OPG foi quantificada por estereologia. A razão do volume ósseo (BV/TV), a espessura trabecular (Tb.Th), a separação trabecular (Tb.Sp) e o número trabecular (Tb.N) foram analisados por microtomografia (Micro-TC). Resultados: Vv de imunoexpressão de RANKL e OPG foram, respectivamente, 53% maiores e 50% menores em animais do grupo IR em comparação com o grupo C ($p<0,05$). A micro-TC mostrou que nos animais do grupo IR houve redução da massa óssea em relação aos animais C. A BV/TV e a Tb.Th foram 33%

e 38% menores, respectivamente, nos animais de IR (p<0,05), enquanto a Tb.Sp foi 37,5% maior nesses animais (p<0,05). Conclusão: A radiação ionizante em dose única, seguida de extrações dentárias, promoveu ORN em osso mandibular de ratos, juntamente, com alterações nos marcadores de remodelamento ósseo RANK e OPG.

Palavras-chaves: Radioterapia, Osteorradionecrose, Remodelamento ósseo, RANKL, OPG.

1 INTRODUCTION

Patients with head and neck cancer in advanced stages often require multimodal treatment including radiotherapy and chemotherapy, combined or not with surgery.[1] Patients can suffer severe late side effects of radiotherapy such as xerostomia, dysphagia and osteoradionecrosis (ORN).[1,2,3] These adverse effects also occur, even with a lower incidence, with the use of new treatment techniques, such as intensity-modulated radiation therapy and stereotactic radiosurgery.[4,5]

The ORN of the jaws is a late and severe complication of radiation therapy used to treat head and neck tumors (HNT).[5] The ORN is a condition that affects 2-20% of patients undergoing radiotherapy for HNT, often defined as an exposed area of bone that persisted for three or more months, when all other diagnoses were excluded.[6,7] However, over the past 35 years, several authors have attempted to define ORN. Beumer et al.[8] stated that ORN of the jaws occurs when the irradiated bone is exposed in the oral cavity for at least two months in the absence of local neoplastic disease. Marx [9] defined ORN as an area of exposed bone within the irradiated area that is larger than one centimeter, which did not show any evidence of healing for at least six months.

In ORN, some patients have chronic exposure of necrotic bone (signs of bone sequestration), mucosal necrosis, ulceration or persistent pain. Other features include swelling, trismus, paresthesia or anesthesia, no tissue adhesion, orocutaneous fistula and pathological fracture.[10] Hyperbaric oxygen therapy and mandibulectomy with bone grafts are considered standard treatment approaches for ORN of the jaws.[11,12]

Physiological bone remodeling is a highly coordinated process responsible for bone resorption and formation, and is necessary to repair damaged bone and to maintain mineral homeostasis.[13] The discovery of the importance of the RANK/RANKL/OPG system in the regulation of bone resorption has led to great advances in the understanding of bone remodeling. It has been known that the osteoblastic stromal cells regulate the formation of osteoclasts and that they perform this activity through the super family

members of the tumor necrosis factor (TNF): receptor activator of nuclear factor-Kappa B (RANK), receptor activator of nuclear factor- kappa B ligand (RANKL) and osteoprotegerin ligand (OPG). The combination RANK/RANKL regulates osteoclast formation (osteoclastogenesis), activation and survival in normal bone remodeling and in a variety of pathological conditions characterized by increased bone metabolism. OPG protects bone excessive resorption by binding to RANKL, preventing it from binding to RANK. Thus, the relative concentration of RANKL and OPG in bone is a major determinant of bone mass and strength.[14, 15]

The precise mechanisms involved in the etiology of ORN are not fully understood yet. Animal models of ORN have been somewhat successful in clarifying the mechanisms involved with the disease. It is known that dental trauma, for example dental extractions, at an irradiated area can increase the incidence of ORN in animals, like in humans.[8,16,17] Thus, the aim of this study was to evaluate the effect of radiation on bone remodeling of the mandibular region submitted to radiotherapy followed by extraction of mandibular molar teeth in rats. The focus was the morphometric study of the irradiated jaw and the involvement of RANK/RANKL/OPG protein markers in the bone remodeling process.

2 MATERIAL AND METHODS

2.1 ANIMALS

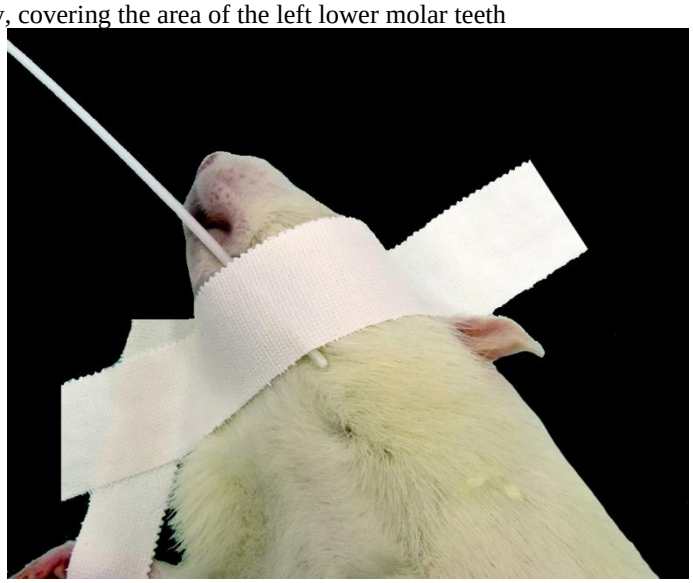
All procedures with animals were performed in accordance with the guidelines agreed by the "Care and Use of Laboratory Animals" (US National Institutes of Health, revised in 1996). All experimental protocols were approved by the Committee on Use and Care of Laboratory Animals of the Fluminense Federal University, Rio de Janeiro, Brazil (protocol number CEUA-716/2016).

Six month old male Wistar rats weighting between 346 and 424 g were kept under controlled conditions (12 h light/dark cycles, 21±2 ºC, and humidity 60 ± 10%) and had free access to food and water. Rats were obtained from the Bioterium of the Department of Radiological Sciences, at Rio de Janeiro State University (DCR/UERJ). The body mass (BM) was assessed weekly (Monday, 8:00) throughout the experiment. A total of 20 rats were used for the entire study. The animals were divided into two groups each containing ten rats (n=10): irradiated group (IR) and control group (C). Each split between micro-CT analysis (n=5) and sterology (n=5). The distribution of animals was performed randomly.

Irradiation

Before irradiation, rats of the IR group were anesthetized with ketamine/xylazine (40 mg/kg Ketamine plus 5 mg/kg Xylazine, intramuscular - IM). Irradiation was performed with an Ir-192 high dose rate (HDR) brachytherapy source, with a nominal activity of 10 Ci, delivering dose at a rate >12Gy/h. Sterile plastic catheter (Alpha Omega Services, Long Beach, CA) was positioned and maintained with adhesive tape along of the skin over the lateral side of the left mandibular body, covering the area of the left lower molar teeth (Figure 1). A single dose of radiation was applied with an HDR remote afterloading system (GammaMed 12it; Varian Medical Systems, Charlottesville, Inc, VA) delivering 20 Gy at the corresponding area of the left lower molar teeth, according to the treatment planning. Animals of group C underwent the same procedures, including anesthesia, but without being irradiated.

Figure 1: HDR plastic catheter positioned and maintained along of the skin over The lateral aspect of the left mandibular body, covering the area of the left lower molar teeth



2.2 DENTAL EXTRACTIONS

On the seventh day after brachytherapy of the IR animals, all animals (IR and C groups) underwent a traumatic extraction of their left mandibular teeth, using specific pliers for tooth extraction. For the procedure, the animals were anesthetized (40 mg/kg Ketamine plus 5 mg/kg Xylazine, IM) and extreme care was taken to avoid breaking the roots of the teeth from the crown. The management of postoperative pain was performed with ibuprofen (Medley Pharmaceuticals Ltd., Brazil) at a dose of 15 mg/kg/day given orally by gavage for three days.

Euthanasia and jaws extraction

Twenty one days after dental extractions (28 days after brachytherapy), the animals received a lethal dose of anesthetic for euthanasia (300 mg/kg Ketamine plus 30 mg/Kg Xylazine, intraperitoneal). The jaws extraction surgical procedure proceeded as follows: dermal and oral disinfection, mouth opening, detaching the gingiva around the second left mandibular molar and extraction of the tooth and dissected for analysis.

2.3 PREPARATION OF THE MANDIBULAR SAMPLES

Five left hemimandibles from each animal from each group (IR and C) were cleaned using saline solution (0.9% NaCl), dried, and separated for micro-CT analysis. Other five left hemimandibles from each animal from each group (IR and C) were first fixed in 10% buffered formalin for 72 hours. Thereafter, they were decalcified with Ana Morse solution (equal parts of sodium citrate and formic acid), which was renewed every 48 hours and daily agitated to speed up decalcification. After complete decalcification samples were separated for light microscopy analysis.

Histopathological analysis

The five decalcified left hemimandibles of each group (C and IR) were sectioned in the mesiodistal direction at the area of the left lower molar teeth, forming two bands (buccal and lingual), that were embedded in paraffin. Histological paraffin sections were obtained from serial 5 µm cuts from two points of the mandibles in the frontal direction (forward molars and at the extraction zone) using a microtome (Laborana, Sao Paulo, Brazil). A light microscopy (Nikon Eclipse E200, Nikon Instruments Inc., Japan) was used for histopathological, stereological and immunohistochemical analysis. For histopathological analysis, the sections were stained with hematoxylin and eosin (HE).[18,19]

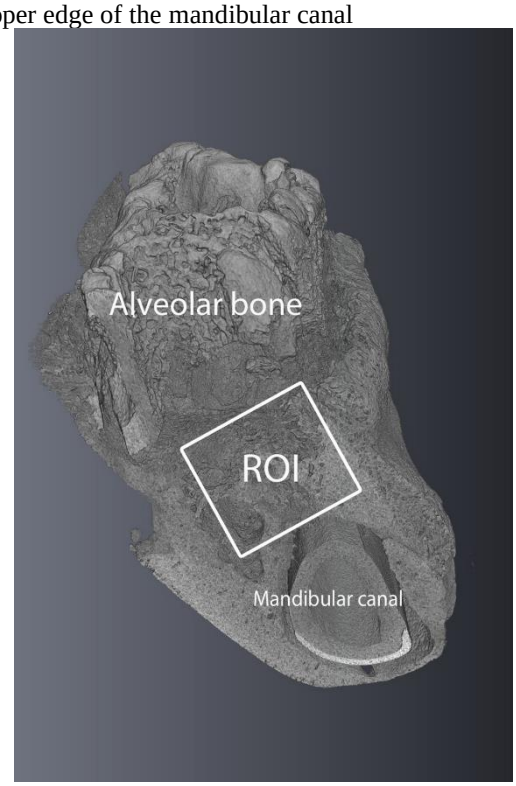
2.4 MICRO-CT ANALYSIS

The samples that were not embedded in paraffin and not decalcified were analyzed by Micro-CT. The images were acquired using a desktop Micro-CT system from Skyscan 1172 Bruker (Kontlich, Belgium) with an final isotropic resolution of 4.4 µm per voxel, at camera binning 2x2, 70 kV accelerating voltage, 142 µA current, with a 0.5-mm aluminum filter placed in front of . The samples were rotated by a total of 180° about its vertical axis at a step size of 0.40°, with exposure time of 1100 ms per projection, taking an average of 4 frames, making a total of 4400 ms per projection. The total scan time per sample was approximately 40 min. The images were reconstructed with NRecon v.1.6

software (Bruker) using a filtered back-projection algorithm for cone-beam setup with the following parameters: ring artifact correction of 7, beam hardening correction of 40% and smoothing of 2 (Gaussian).

For quantification, a region of interest (ROI) was chose between the apexes of the left lower molar teeth and the upper edge of the mandibular canal. The area contained only trabecular bone, with dimensions of 100 x 100 x 300 pixels³ (WxDxH), which equals 0.44 x 0.44 x 1.32 mm³ (Figure 2).

Figure 2: 3D micro-CT reconstruction showing ROI for analysis: located between the apexes of the left lower molar teeth and the upper edge of the mandibular canal



The main parameter extracted from the three-dimensional (3D) image was the bone volume fraction, calculated by bone volume (BV) and total volume (TV), (BV/TV). From BV/TV ratio, three others parameters were calculated: trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and the trabecular number (Tb.N).[20]

Three-dimensional reconstructions and volume analysis were accomplished using VSGAvizo®Fire software platform (VSG, Burlington, MA). BV/TV parameter, which is the ratio of bone volume to the volume of the whole examined sample, calculated by the number of voxels corresponding to the bone, divided by the total number of image voxels. The Tb.Th was computed based on the calculation of the diameter of the largest sphere contained within the structure of the trabeculae. The Tb.Sp was calculated through the

same procedure as Tb.Th, however, it is applied to the complement of the trabecular bone structure in the image (region void between the trabeculae). Tb.N was calculated by equation (1).

$$(1)\ Tb.N = \frac{BS}{2BV}$$

Since BS is the surface of trabecular bone, and BV is the bone volume in ROI. The bone surface is computed by counting voxels representing the edge of the trabeculae.

2.5 IMMUNOHISTOCHEMICAL ANALYSIS

Antigen retrieval was performed with citrate buffer pH 6.0, then endogenous peroxidase was quenched with hydrogen peroxide 3%, and finally, non specific binding was inhibited with phosphate buffered saline/bovine serum albumin 5%. Slides were incubated with primary monoclonal antibodies anti-RANK (sc-52951, 1:100 dilution, Santa Cruz Biotechnology, Dallas, Texas - USA), anti-RANKL (ab-45039, 1:50 dilution, Abcam discover more, Cambridge - UK) and anti-OPG polyclonal antibody (sc-8468, 1:50 dilution, Santa Cruz Biotechnology) in a humid chamber overnight at 4° C. After incubation, slides were washed in PBS pH 7.4 (twice, 5 min each). Secondary antibody conjugated with peroxidase was added (strept complex AB/HRP duct, mouse/rabbit/goat, Dako Carpinteria, CA, 1:500) for 30 minutes at 37°C. The reaction was amplified with a biotin-streptavidin system (strept Complex AB/HRP duct, mouse/rabbit/goat, Dako, 1:500) for 30 minutes. The reaction was visualized after incubation with 3,3' diaminobenzidine tetrachloride-8-(K3466, DakoCytomation, Glostrup, Denmark), and sections were counterstained with Mayer hematoxylin. The positive controls were obtained from bone tissue to RANK, RANKL and OPG, and negative controls were obtained by omitting primary antibodies.

2.6 STEREOLOGY

The volume density (Vv) of immunostained cells for RANK, RANKL and OPG was estimated by counting points with a test system consisting of 36 test points in at least ten different fields per animal, eg. 36 x 10 x 5 = 1800 dots-per test group. Whereas the lower Vv obtained was 8.0%, the stereological design to estimate the Vv follows a standard error acceptable calculated 0.049 and $p<0.05$, based on Pt = 0.453 * equation (1

- Vv) / (Vv * E2) (http://www.ou.edu/research/electron/bmz5364/calc-stereology.html). The test system was produced with the web-based system by STEPanizer (www.stepanizer.com). [21]

Statistics

Data are shown as mean (M) and standard deviation (SD). After the test for normality, Student t test was used to compare data between C and IR group (Prism version 5.03, GraphPad Software, La Jolla, CA, USA). For all analyses, a p-value of < 0.05 was considered statistically significant.

3 RESULTS

There were no deaths after radiotherapy and tooth extractions. Animals of group IR showed alopecia on the exposed region to radiation, demonstrating clinical manifestations of irradiation. C and IR animals showed no significant changes in body weight during the experiment and no statistical difference between the groups was observed (375.6 ± 21 g for C animals and 386.5 ± 22 g for IR animals).

3.1 HISTOPATHOLOGY

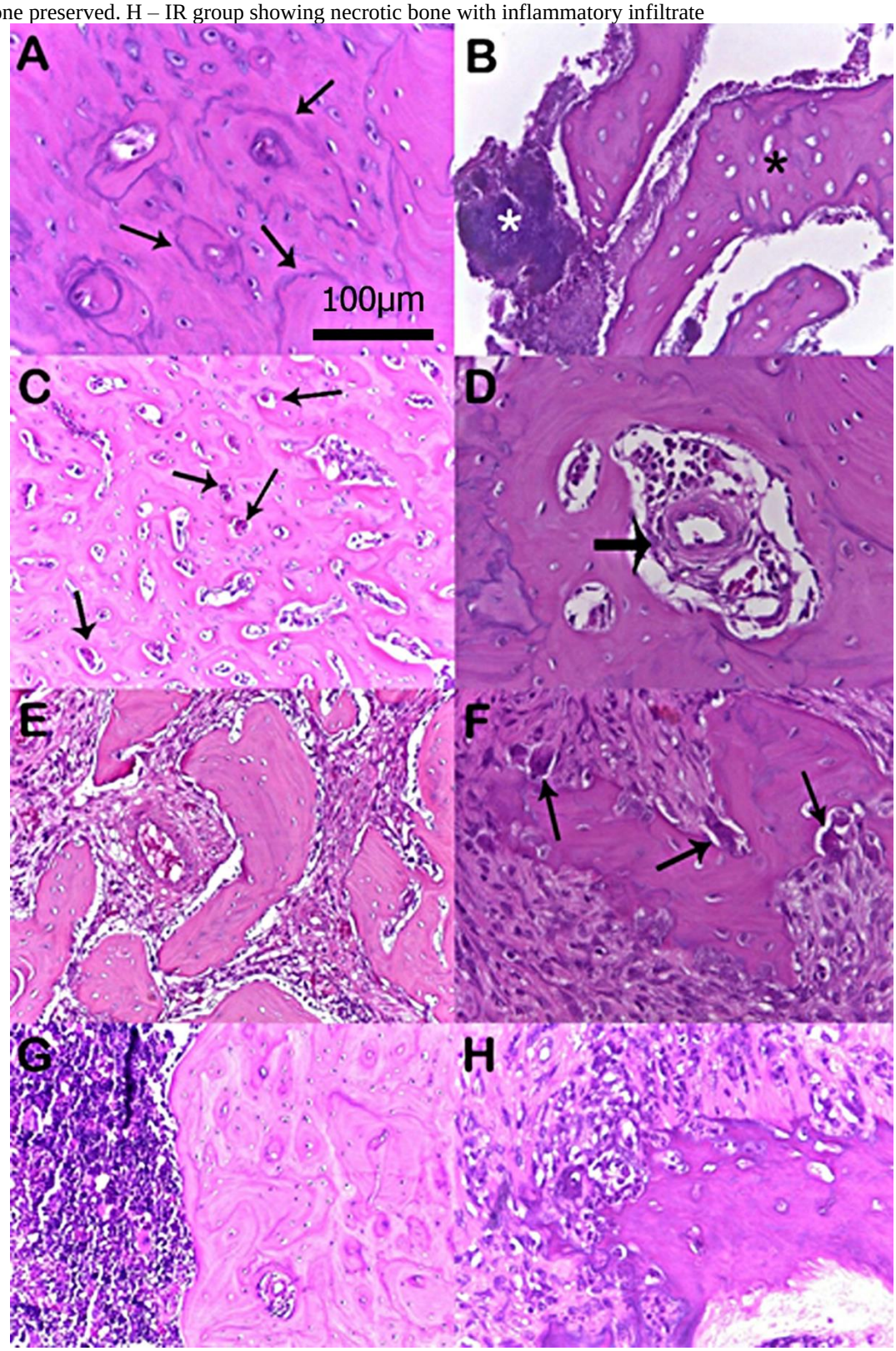
The histological features are shown in Table 1 and describe (or not) the presence of bone necrosis, characterizing, thus, ORN in the jaws in animals treated by brachytherapy.

Table 1 – Histological features of bone tissue in the mandibles of IR animals treated by brachytherapy

Bone tissue	Vitalized	Devitalized
Vascularization	Normal	Reduced
Osteoblasts and Osteoclasts	With activity	Without activity
Bacteria	No	Yes
Bone Marrow	Without fibrosis	With fibrosis
Inflammation	No	Yes
Vascular tissue	Without thickening	With thickening

IR group showed significant histopathological changes not found in the C group as the presence of focal areas of devitalized bone, vascular changes, the presence of bacteria and the presence of inflammatory infiltrate associated with bacterial colony. Moreover, we observed in both groups intense presence of basophilic reverse lines, which indicates recent bone deposition area. Thus, our study was effective in promoting bone damage in the animals treated with brachytherapy compatible with ORN (Figure 3).

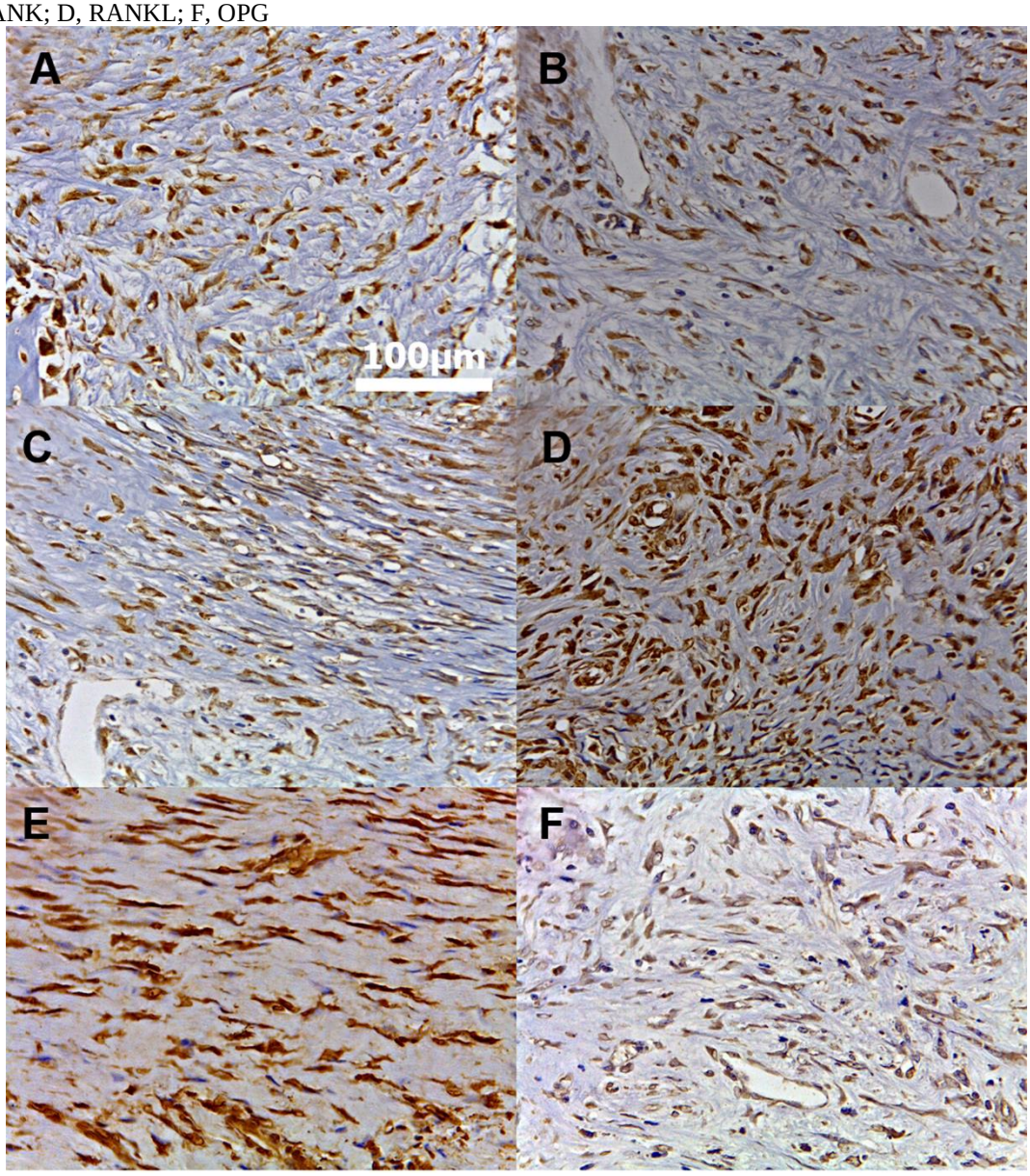
Figure 3: Photomicrographs of the mandibular bone (same magnification, HE stain) showing the histopathological features to characterize or not ORN in animals treated by radiation. A – Control group with presence of basophilic reverse lines (arrows) and absence of bacteria. B – IR group showing areas of necrotic bone (black asterisk) and presence of bacteria (white asterisk). C – Control group showing bone tissue with rich vascularization (arrows). D – IR group showing a vessel with thickening of its wall (arrow). E – Control group with presence of osteoblastic rimming (area of bone deposition). F – IR group with numerous osteoclasts (arrows). G – Control group showing marrow bone without fibrosis and compact bone preserved. H – IR group showing necrotic bone with inflammatory infiltrate



3.2 IMMUNOHISTOCHEMISTRY AND STEREOLOGY

Immunostaining for RANK, RANKL and OPG in the region of the left mandibular molar teeth of C and IR animals are shown in Figure 4.

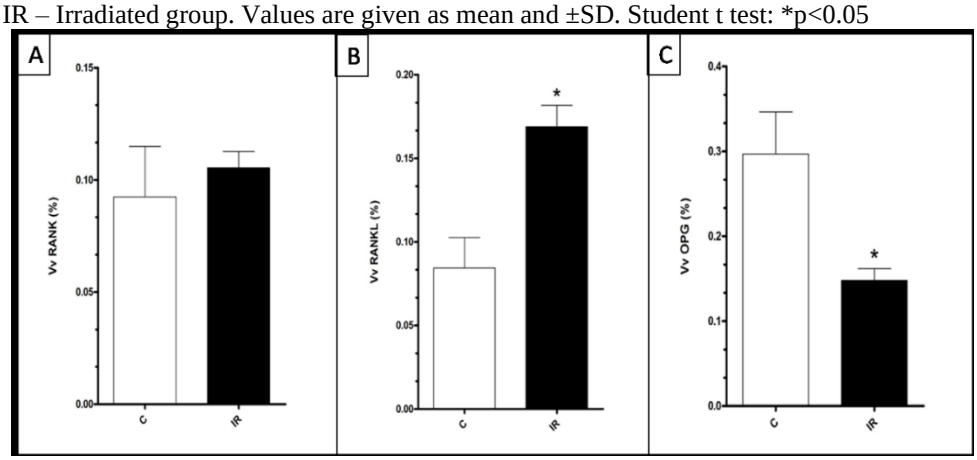
Figure 4: Photomicrographs of the mandible bone showing immunostaining of RANK, RANKL and OPG in both control (C) and irradiated (IR) groups. C animals: A, RANK; C, RANKL; E, OPG. IR animals: B, RANK; D, RANKL; F, OPG



There was no difference in Vv of immunostaining for RANK among C and IR animals (Figure 5A). However, IR group showed 53% higher of RANKL staining compared with C animals ($p<0.05$) (Figure 5B). The opposite occurred with OPG, where IR group showed 50% lower ($p<0.05$) of immunostaining compared with group C (Figure 5C). The increase in immunostaining of RANKL followed by a decrease of OPG in animals that were treated with radiotherapy (IR group) suggests a possible increase in

osteoclastogenesis in this group, resulting in loss and reduction of bone mass in these animals.

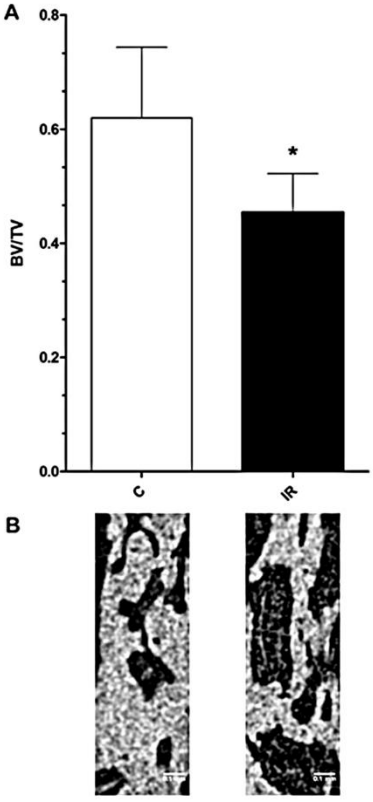
Figure 5 – Volume density (Vv) of: A) RANK immunostaining in mandible bone; B) Volume density (Vv) of RANKL immunostaining in mandible bone; C) OPG immunostaining in mandible bone. C – Control group; IR – Irradiated group. Values are given as mean and ±SD. Student t test: *p<0.05



3.3 MICRO-CT

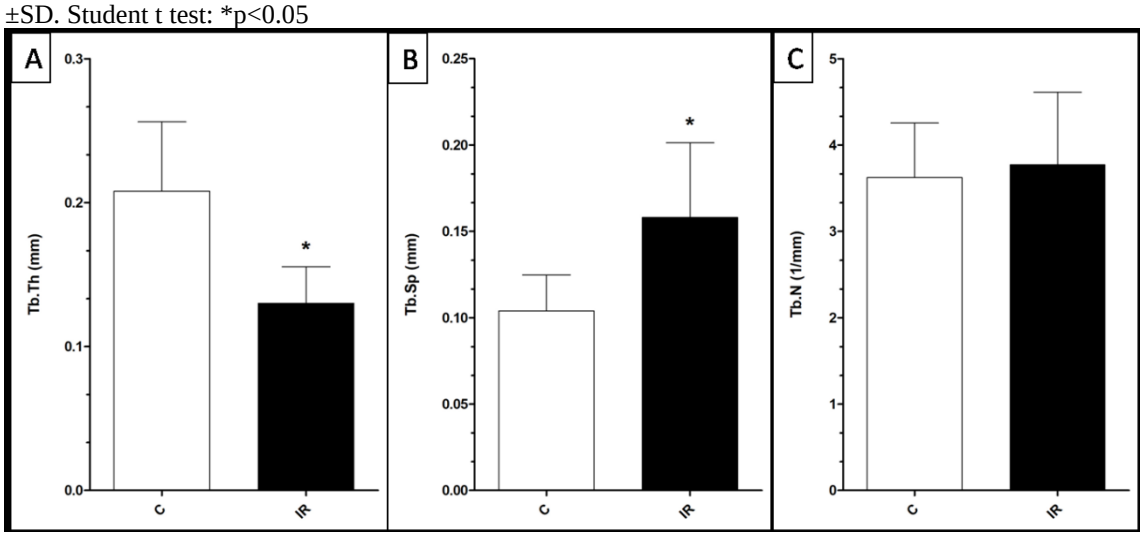
The Micro-CT analysis showed that bone volume ratio (BV/TV) was 33% lower ($p<0.05$) in IR animals than in C animals (Figure 6).

Figure 6 – Micro-CT analysis of bone volume ratio (BV/TV). C – Control group; IR – Irradiated group. Values are given as mean and ±SD. Student t test: *p<0.05



Similarly, the trabecular thickness (Tb.Th) was 38% lower ($p<0.05$) in IR animals. IR animals showed a thickness of 0.13 ± 0.02 mm compared to those 0.21 ± 0.05 mm of C animals (Figure 7A). As a result of BV/TV and Tb.Th reduction in IR group, there was an increase in average separation of the trabecular bone (Tb.Sp) in these animals. IR group showed Tb.Sp of 0.16 ± 0.04 mm, showing a distance among bone septa 37.5% higher ($p<0.05$) than C animals, which showed a mean separation of 0.10 ± 0.02 mm (Figure 7B). There was no difference in the average trabecular number (Tb.N) between IR and C groups (Figure 7C).

Figure 7 – Micro-CT analysis of average: A) trabecular thickness (Tb.Th); B) trabecular separation (Tb.Sp); C) trabecular number (Tb.N). C – Control group; IR – Irradiated group. Values are given as mean and ±SD. Student t test: *p<0.05



4 DISCUSSION

ORN is defined as a sequel resulting from radiotherapy characterized by the loss of oral mucosa and exposure of necrotic bone tissue.[1] The ORN of the mandible is not a rare consequence of irradiation applied in this area for treatment of malignancies. Three etiological factors should be considered: intensity of radiotherapy; trauma of the structures affected by radiation; and infection of the jaw bone by the oral flora. There is no doubt that the greatest adjunct to the development of ORN is the dental extraction procedure, just before or after the radiotherapy treatment of neoplasms belonging to the head and neck region.[22, 23] Dental extractions increase the impact of cell effects of radiation, such as absence of bone healing, increase of osteoclast activity and decrease of bone vascularization. The ORN is normally a late effect and may arise in the first year or even many years after radiotherapy because no ideal bone healing occurs.[17]

Animal models for development of mandibular ORN disease show similar bone damage like in humans. Brachytherapy model incorporating dental extractions after

radiation and using a higher radiation dose have successfully demonstrated reproducible radiogenic mandibular bone damage analogous to the clinical ORN. [24, 25] A model of maxillary ORN also exists but shows subclinical response to radiation and lower incidence of bone damage, probably because of the differences in the maxillary bone and microenvironment of the maxilla compared with the mandible.[16, 26]

In this study it was used a rat model that received a dose of 20 Gy using Ir-192 HDR brachytherapy. This model successfully created bone damage like ORN disease. Using the quadratic linear model and a α/β ratio of 3 for late normal tissue complications, acute exposure of 20 Gy is equivalent to a total dose of 92 Gy when the irradiation scheme is done with fractional doses of 2 Gy, representing a clinically relevant radiation dose.

The hypothesis suggested by this study to explain the bone changes is that radiation at high doses impairs the healing process of the tooth socket, due to the alteration of the molecules involved with bone remodeling process, such as RANKL and OPG. The balance between RANKL and OPG is crucial to osteoclastogenesis and bone metabolism.[27] The ligand RANKL, found on the osteoblast surface, interacts with RANK, found on the osteoclast precursor cell surface, promoting osteoclastogenesis. The OPG blocks this interaction and inhibits the osteoclast formation.[27,28] Irradiation causes an increase in the cytokines involved with osteoclastogenesis, including TNF-α, IL-1β and IFN-γ and these pro-inflammatory cytokines induce the formation of RANKL, promoting osteoclastogenesis with subsequent loss of bone mass in the irradiated area.

This work corroborates with this evidence, since brachytherapy altered the bone remodeling by interfering with the balance of the RANK/RANKL/OPG system, significantly reducing OPG and increasing RANKL immunostaining in the IR group compared to the C group. These changes would lead to an increased activity of osteoclastic pathway, thereby inducing a reduction in the bone mass in the IR group. There was no difference in immunostaining of RANK between IR and C groups, suggesting that the cause of the ORN promotion is the activation of osteoclastogenesis, that is regulated by osteoblasts cells from RANKL.

The Micro-CT analysis corroborates with the immunohistochemical analysis, suggesting an increase in osteoclastogenesis in animals undergoing brachytherapy. In fact, these animals also showed a reduction in the bone mass in the area where the catheter covered with the radioactive source (left mandibular molar teeth), as evidenced by the reduction of BV/TV and Tb.Th and the increase of Tb.Sp. Thus, there is a strong indicative that the molecules involved in osteoclastogenesis, consequently in bone

remodeling (RANKL and OPG), are modulated during radiotherapy, leading to changes that result in bone loss, similar to what happens in the ORN.

Some studies using Micro-CT analysis had highlighted significant reduction in the total volume of bone and in the cortical thickness after irradiation, and showed a decrease of intraosseous vascularization.[29,30] Likewise, in the present study, through the Micro-CT technique, it was possible to evidence the bone loss by observing the reduction of the of trabecular bone thickness in the IR group. This bone loss can be explained by the effect caused by radiation in healing and bone remodeling.

The hypovascularization observed in the histopathological results may have contributed to the inflammatory process and the changes in bone metabolism. Previous studies have already demonstrated the effect of radiotherapy on vascularization.[30] The radiation arteritis leads to the development of a hypocellular, hypovascular and hypoxic environment, which results in a pathological outcome.[9,31,32] Histologically, a loss of vascular structures after irradiation is described, resulting in swelling and vacuolation of the endothelial cell cytoplasm.[33] The findings in our histopathological studies show that there is a thickening of the arterial wall in specific focal areas of the slides examined in the irradiated group, which did not happen in the control group. Still in the IR group, focal areas of devitalized bone with absence of osteocytes and the presence of bacteria and associated inflammatory infiltrate were observed. These results corroborates with previous experiments from literature. [34]

A number of side effects of radiation therapy for head and neck cancers (eg. xerostomia) have long been thought to be due to the detrimental impact on salivary gland (SG) function. When SG function is diminished, saliva flow and protective effect are decreased, and thus oral pathogens have greater opportunity to colonize.[35] The removal of teeth would allow ready entry of any oral pathogen and an immune response to such infection can lead to inflammation and subsequent localized bone loss.[23] In this regard, it is interesting that bacteria were noted in a section of IR mandibular bone but not in Control bone. This may indeed suggest changes in oral flora post IR, ie. secondary to salivary gland perturbation, and that this is the causal basis for the changes in expression and bone loss [35,36], as noted in other animal models. [37,38]

The reduction of the side effects resulting from radiotherapy treatment is extremely important for the quality of life of an oncology patient. That is why it is important to know the genesis of the pathology to try to mitigate its effects.

The management of ORN can be complex and often requires a multimodality approach. Nonsurgical treatments with or without adjunct measures and surgical interventions have all been employed on the basis of staging of the disease process.[39, 40,41] This study will assist in the designing of intervention programs for irradiation-induced bone loss.

Conclusion

Brachytherapy in a high single dose (20 Gy) is able to induce bone damage in the jaw bone in rats after post radiation dental extraction. There is a positive evidence between high dose rate radiation and decreased bone mass from the interference with the bone remodeling regulatory factors, specifically, the RANKL and OPG. These findings apply new knowledge to better understanding the bone remodeling process in the ORN disease.

ACKNOWLEDGMENTS

Irradiation was performed at the University Hospital Clementino Fraga Filho of Rio de Janeiro Federal University (HUCFF/UFRJ).

Oral Pathology Laboratory of the Fluminense Federal University - Nova Friburgo under coordinator of Dr. Rebeca Azevedo.

PhD Regina Cely Barroso (UERJ) and PhD Carlos Eduardo Veloso de Almeida (UERJ) for your contributions in data interpretation.

This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

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